CLAIMS:

1. A PCR reaction mixture comprising
a target nucleic acid
PCR reagents,
oligonucleotide primers configured for amplifying the target nucleic acid, and
a dsDNA binding dye having a percent saturation of at least 50%.

2. The PCR reaction mixture of claim 1 wherein the dsDNA binding dye has a percent saturation of at least 80%.

3. The PCR reaction mixture of claim 1 wherein the dsDNA binding dye has a percent saturation of at least 90%.

4. The PCR reaction mixture of claim 1 wherein the dsDNA binding dye has a percent saturation of at least 99%.

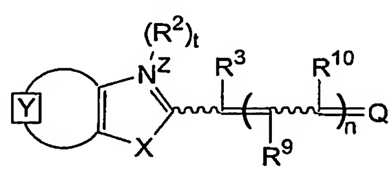
5. The PCR reaction mixture of claim 1 wherein the dye has an excitation maximum between about 410 and 460 nm, and an emission maximum between about 450 and 500 nm.

6. The PCR reaction mixture of claim 1 wherein the dye has an excitation maximum between about 430 and 460 nm, and an emission maximum between about 460 and 490 nm.

7. The PCR reaction mixture of claim 1 wherein the dye is a cyanine dye capable of detecting heterozygotes during melting temperature analysis.

8. The PCR reaction mixture of claim 7 wherein the cyanine dye has a pyridinium, pyrimidinium, or quinolinium core structure.

9. The PCR reaction mixture of claim 7 wherein the cyanine dye is a compound having the formula:



wherein .

the moiety $\boxed{Y}$ represents an optionally-substituted fused mono or polycyclic aromatic or nitrogen-containing heteroaromatic ring;

X is oxygen, sulfur, selenium, tellurium, or a group selected from $C(CH_3)_2$ and NR^1, where R^1 is hydrogen or alkyl;

R^2 is alkyl;

$t = 0$ or 1;

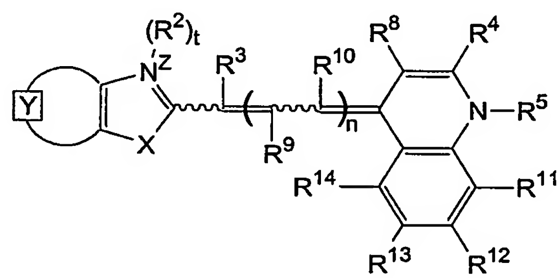
Z is a charge selected from 0 or 1;

R^3, R^9, and R^{10} are each independently selected from hydrogen and alkyl;

$n = 0$, 1, or 2; and

Q is a heterocycle, such as a pyridinium, a pyrimidinium, a quinolinium, or a purinium, each of which may be optionally substituted.

10. The PCR reaction mixture of claim 7 wherein the cyanine dye is a compound having the formula:



wherein

the moiety $\boxed{Y}$ represents an optionally-substituted fused mono or polycyclic aromatic or nitrogen-containing heteroaromatic ring;

X is oxygen, sulfur, or a group selected from $C(CH_3)_2$, and NR^1, where R^1 is hydrogen or C_{1-6} alkyl;

R^2 is alkyl;

$t = 0$ or 1;

Z is a charge selected from 0 or 1;

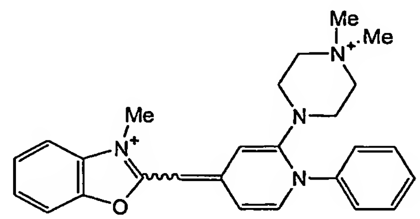
R^3, R^9, and R^{10} are each independently selected from hydrogen and alkyl;

$n = 0$, 1, or 2; and

R^4, R^5, R^8, R^{11}, R^{12}, R^{13}, and R^{14} are as described herein for Formula I, providing that R^4 is a moiety with a molecular weight of less than about 115, or illustratively a molecular weight of less than about 105.

11. The PCR reaction mixture of claim 7 wherein the cyanine dye is LightCycler Green.

12. The PCR reaction mixture of claim 7 wherein the cyanine dye is a compound having the formula:



13. The PCR reaction mixture of claim 1 wherein the dye is selected from the group consisting of JO-PRO™-1, GelStar®, SYTO® 44, SYTO® 45, POPO™-3, SYTO® 12, TOTO™-3, SYTO® 16, SYTOX® Blue, Thiazole Orange, YOYO®-3, SYTO® 43, SYTO® 11, SYTO® 13, SYTO® 15, BOBO™-3, LO-PRO™-1, SYTO® 23, SYTO® 20, BOBO™-1, POPO™-1, G5, H5, D6, E6, P6, R6, Y6, Z6, and D8.

14. The PCR reaction mixture of claim 1 wherein the dye is present at a concentration of at least 50% of the maximal concentration compatible with PCR.

15. The PCR reaction mixture of claim 1 wherein the dye is present at a concentration of 90-100% of maximal concentration compatible with PCR.

16. The PCR reaction mixture of claim 1 wherein the dye is present at a concentration of no more than 20% of the maximal concentration compatible with PCR.

17. A method of genotyping comprising the steps of:

amplifying a target nucleic acid in the presence of a dsDNA binding dye having a percent saturation of at least 50%,

melting the amplified target nucleic acid to generate a melting curve, and

identifying the genotype from the melting curve.

18. The method of claim 17 wherein the melting curve is generated using a fluorimeter having an excitation range of 450-490 nm and an emission detection range of 510-530 nm, and the dye has an excitation maximum in a range of 410-465 nm and an emission maximum in a range of 450-500 nm.

19. The method of claim 18 wherein the dye's excitation maximum is in the range of 430-460 nm and emission maximum is in the range of 450-500 nm.

20. The method of claim 17 wherein the target nucleic acid comprises a single nucleotide polymorphism, and the identifying step comprises identifying resultant heteroduplexes and homoduplexes.

21. The method of claim 17 wherein the melting step occurs subsequent to amplification.

22. The method of claim 17 wherein the melting step occurs during amplification.

23. A method of mutation scanning comprising the steps of:

(a) adding a dsDNA binding dye having a percent saturation of at least 50% to a sample comprising a target nucleic acid,

(b) amplifying the target nucleic acid in the presence of the dsDNA binding dye,

(c) melting the amplified target nucleic acid to generate a melting curve,

(d) repeating steps (b) and (c) on second sample to obtain a second melting curve, and

(e) comparing the melting curves.

24. A method of PCR analysis comprising the steps of:

mixing a dsDNA binding dye having a percent saturation of at least 50% with a sample comprising a target nucleic acid and primers configured for amplifying the target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye, and

monitoring fluorescence of the dsDNA binding dye.

25. The method of claim 24 further comprising the steps of

generating a melting curve for the target nucleic acid,

normalizing the magnitude of the melting curve,

repeating the mixing, amplifying, generating, and normalizing steps with at least one additional target nucleic acid, and

comparing the normalized melting curves.

26. The method of claim 25 further comprising the step of plotting the temperature difference between the normalized curves.

27. The method of claim 25 further comprising the step of temperature shifting the melting curves by superimposing a portion of each curve.

28. The method of claim 27 further comprising the step of plotting the temperature difference between the temperature shifted curves.

29. The method of claim 24 or 25 wherein the dye is selected from the group consisting of LC Green, Gel Star, and SYTO® 16.

30. The method of claim 25 wherein the dye is selected from the group consisting of PO-PRO™-1, JO-PRO™-1, BO-PRO™-1, SYTO® 44, SYTO® 45, YO-PRO®-1 POPO™-3, SYTO® 12, TOTO™-3, SYTOX® Blue, Thiazole Orange, YOYO®-3, SYTO® 43, SYTO® 11, SYTO® 13, SYTO® 15, BOBO™-3, LO-PRO™-1, SYTO® 23, TO-PRO®-1, SYTO® 20, BOBO™-1, POPO™-1, G5, H5, D6, E6, P6, R6, Y6, Z6, and D8.

31. The method of claim 24 wherein the fluorescence is monitored during amplification.

32. The method of claim 24 wherein the fluorescence is monitored during melting curve analysis subsequent to amplification.

33. The method of claim 24 wherein the sample further comprises a probe configured to hybridize to the target nucleic acid, said probe labeled with an acceptor dye to accept fluorescent resonance energy transfer from the dsDNA binding dye, and further comprising the step of monitoring fluorescence from the acceptor dye.

34. The method of claim 24 wherein the target nucleic acid is no greater than 100 bp.

35. The method of claim 34 wherein the target nucleic acid is no greater than 50 bp and comprises only a single melting domain.

36. The method of claim 24 wherein the target nucleic acid comprises a variable melting domain and an invariant melting domain.

37. The method of claim 36 further comprising the steps of generating a melting curve for the target nucleic acid,

repeating the mixing, amplifying and generating steps with at least one additional target nucleic acid,

using the invariant melting domain for temperature axis adjustment, and

comparing the melting curve for the target nucleic acid with the melting curve for the additional target nucleic acid.

38. The method of claim 24 wherein the dye is selected from the group consisting of LC Green, PO-PRO™-1, JO-PRO™-1, and BO-PRO™-1.

39. The method of claim 24 wherein the amplifying and monitoring occur in a closed tube, and no reagents are added to the tube subsequent to initiation of amplification.

40. The method of claim 24 wherein the monitoring step occurs subsequent to the amplifying step and comprises melting curve analysis.

41. The method of claim 24 wherein the monitoring step occurs during amplification.

42. The method of claim 41 further comprising the step of performing post-amplification melting curve analysis.

43. A method of PCR analysis comprising

cycling a PCR mixture of claims 1-17 between at least an annealing temperature and a denaturation temperature to amplify the target nucleic acid,

generating a melting curve for the target nucleic acid, and

using the melting curve to determine whether the target nucleic acid has the same sequence as a second nucleic acid.

44. The method of claim 43 wherein the cycling the generating steps occur in a closed tube, and no reagents are added to the tube subsequent to initiation of amplification.

45. A method of PCR analysis comprising the steps of:

mixing a dsDNA binding dye with a sample comprising a target nucleic acid and primers configured for amplifying the target nucleic acid,

amplifying the target nucleic acid in the presence of the dsDNA binding dye,

monitoring fluorescence of the dsDNA binding dye,

generating a melting curve for the target nucleic acid,

normalizing the melting curve,

repeating the mixing, amplifying, normalizing, and generating steps with at least one additional target nucleic acid, and

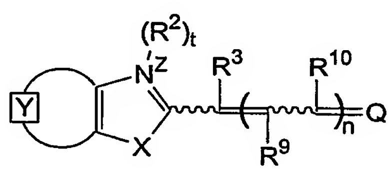
comparing the normalized melting curves.

46. The method of claim 45 further comprising the step of plotting the temperature difference between the normalized curves.

47. The method of claim 45 further comprising the step of temperature shifting the melting curves by superimposing a portion of each curve.

48. The method of claim 47 further comprising the step of plotting the temperature difference between the temperature shifted curves.

49. A compound having the formula:



wherein

the moiety $\boxed{Y}$ represents an optionally-substituted fused monocyclic or polycyclic aromatic ring or an optionally-substituted fused monocyclic or polycyclic nitrogen-containing heteroaromatic ring;

X is oxygen, sulfur, selenium, tellurium or a moiety selected from $C(CH_3)_2$ and NR^1, where R^1 is hydrogen or C_{1-6} alkyl;

R^2 is selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, aryl, aryl(C_{1-2} alkyl), hydroxyalkyl, alkoxyalkyl, aminoalkyl, mono and dialkylaminoalkyl, trialkylammoniumalkyl, alkylenecarboxylate, alkylenecarboxamide, alkylenesulfonate, optionally substituted cyclic heteroatom-containing moieties, and optionally substituted acyclic heteroatom-containing moieties;

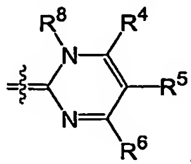
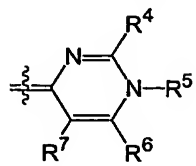
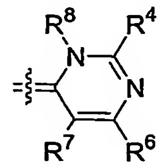
t = 0 or 1;

Z is a charge selected from 0 or 1;

R^3, R^9, and R^{10} are each independently selected from the group consisting of hydrogen and C_{1-6} alkyl;

n = 0, 1, or 2; and

Q is an heterocycle selected from the group of structures consisting of:

 ,  , and 

wherein R^4, R^5, R^6, R^7, and R^8 are independently selected from the group consisting of hydrogen, halogen, alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, alkenyl, polyalkenyl, alkynyl, polyalkynyl, alkenylalkynyl, aryl, heteroaryl, alkoxy, alkylthio, and dialkylamino, each of which may be optionally substituted; an acyclic heteroatom-containing moiety or a cyclic heteroatom-containing moiety; a BRIDGE-DYE; and a reactive group; each of which optionally includes a quaternary ammonium moiety.

50. The compound of claim 49 wherein the moiety $\boxed{Y}$ represents an optionally-substituted fused monocyclic or polycyclic aromatic ring selected from optionally substituted benzo and optionally substituted naphtho.

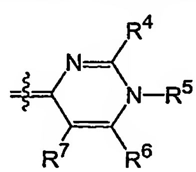
51. The compound of claim 49 wherein the moiety $\boxed{Y}$ represents a benzo or a naphtho having a substituent selected from the group consisting of halo, alkyl, amino, monoalkylamino, dialkylamino, alkylsulfonyl, haloalkylsulfonyl, and optionally substituted phenylsulfonyl.

52. The compound of claim 49 wherein X is oxygen or sulfur.

53. The compound of claim 49 wherein R^2 is selected from the group consisting of C_{1-6} alkyl, C_{3-8} cycloalkyl, aryl, aryl(C_{1-2} alkyl), aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, alkylenesulfonate, optionally substituted cyclic heteroatom-containing moieties, and optionally substituted acyclic heteroatom-containing moieties.

54. The compound of claim 49 wherein R^3, R^9, and R^{10} are each independently selected from the group consisting of hydrogen and methyl.

55. The compound of claim 49 wherein Q is the heterocycle:

 .

56. The compound of claim 49 wherein R^4, R^5, R^6, R^7, and R^8 are independently selected from the group consisting of hydrogen, halogen, thiol, alkyl,

aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammmoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

57. The compound of claim 49 wherein t is 1, n = 0, and at least one of R^4, R^5, R^6, R^7, and R^8 is selected from the group consisting of halogen, thiol, C_{2-6} alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammmoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

58. The compound of claim 57 wherein R^5 is selected from the group consisting of halogen, thiol, C_{2-6} alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammmoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

59. The compound of claim 57 wherein the moiety $\boxed{Y}$ represents a benzo or naphtho having a substitutent selected from the group consisting of halo, alkyl, amino, monoalkylamino, dialkylamino, alkylsulfonyl, haloalkylsulfonyl, and optionally substituted phenylsulfonyl; and X is oxygen or sulfur.

60. The compound of claim 57 wherein R^2 is selected from the group consisting of C_{1-6} alkyl, aryl, aryl(C_{1-2} alkyl), aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, and alkylenesulfonate.

61. The compound of claim 57 wherein R^3, R^9, and R^{10} are each hydrogen; and R^2 is selected from the group consisting of C_{1-6} alkyl, aryl, aryl(C_{1-2} alkyl), aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, and alkylenesulfonate.

62. The compound of claim 57 wherein R^5 is selected from the group consisting of C_{2-6} alkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammmoniumalkyl, piperidino, piperazino, 4-methylpiperazinium-1-yl, and aryl.

63. A method for nucleic acid analysis comprising the steps of

mixing a target nucleic acid that is at least partially double stranded with a dsDNA binding dye having a percent saturation of at least 50% to form a mixture, and

generating a melting curve for the target nucleic acid by measuring fluorescence from the dsDNA binding dye as the mixture is heated.

64. The method of claim 63 further comprising the step of comparing the melting curve for the target nucleic acid with a melting curve for a second nucleic acid.

65. The method of claim 63 wherein the target nucleic acid is a locus of an HLA gene from a first individual and the second nucleic acid is the same locus of an HLA gene from a second individual.

66. The method of claim 65 wherein the melting curve for the target nucleic acid is similar to the melting curve for the second nucleic acid, and further comprising the steps of

generating a melting curve for a mixture of the target nucleic acid and the second nucleic acid, and

comparing the melting curve for the target nucleic acid or the second nucleic acid with the melting curve for the mixture of the target nucleic acid and the second nucleic acid.

67. The method of claim 63 further comprising the steps of

mixing the target nucleic acid and a second nucleic acid to generate a mixture,

adding the dsDNA binding dye,

heating and cooling the mixture,

generating a melting curve for the mixed target nucleic acid and the second nucleic acid, and

comparing the melting curve for the mixed target nucleic acid and the second nucleic acid with the melting curve for the target nucleic acid.

68. The method of claim 63 further comprising the steps of

adding a pair of oligonucleotide primers and amplification reagents to the mixture,

amplifying the mixture,

wherein the amplifying step occurs before the generating the melting curve step.

69. A kit for amplifying a target nucleic acid comprising:

amplification reagents,

oligonucleotide primers configured for amplifying the target nucleic acid, and

a dsDNA binding dye having a percent saturation of at least 50%.

70. The kit of claim 69 wherein the dsDNA binding dye has a percent saturation of at least 90%.

71. The kit of claim 69 wherein the dsDNA binding dye has a percent saturation of at least 99%.

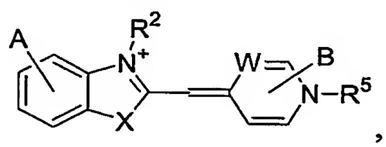
72. The kit of claim 69 wherein the dye is LC Green.

73. The kit of claim 69 wherein the dye is selected from the group consisting of JO-PRO™-1, GelStar®, SYTO® 44, SYTO® 45, POPO™-3, SYTO® 12, TOTO™-3, SYTO® 16, SYTOX® Blue, Thiazole Orange, YOYO®-3, SYTO® 43, SYTO® 11, SYTO® 13, SYTO® 15, BOBO™-3, LO-PRO™-1, SYTO® 23, SYTO® 20, BOBO™-1, POPO™-1, G5, H5, D6, P6, Y6 and D8.

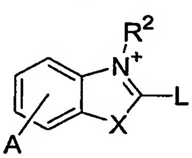
74. The kit of claim 69 wherein the dye has an excitation maximum between about 410 and 460 nm, and an emission maximum between about 450 and 500 nm.

75. The kit of claim 69 wherein the amplification reagents comprise a thermostable polymerase.

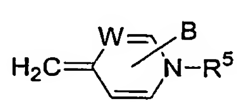
76. A process for preparing a dye having the formula:



the process comprising the step of reacting a compound having the formula:



with a compound having the formula:



wherein:

W is -C(R^8)= or -N=;

A is hydrogen, or A represents one or more substituents each independently selected from the group consisting of alkyl, halo, amino, haloalkyl, alkoxy, haloalkoxy, alkylsulfonyl, haloalkylsulfonyl, arylsulfonyl, alkylthio, arylthio, formyl, alkylcarbonyl, arylcarbonyl, carboxylic acid derivatives, monoalkylamino,

dialkylamino, trialkylammonium, dialkylaminoalkyl, trialkylammoniumalkyl, piperidino, piperazino, each of which may be optionally substituted with alkyl, amino, mono or dialkylaminoalkyl, trialkylammoniumalkyl, or may be optionally quaternized on the nitrogen with an alkyl group;

B is hydrogen, or B represents one or more substituents each independently selected from the group consisting of alkyl, halo, amino, haloalkyl, alkoxy, haloalkoxy, alkylsulfonyl, haloalkylsulfonyl, arylsulfonyl, alkylthio, arylthio, formyl, alkylcarbonyl, arylcarbonyl, carboxylic acid derivatives, monoalkylamino, dialkylamino, trialkylammonium, dialkylaminoalkyl, trialkylammoniumalkyl, piperidino, piperazino, each of which may be optionally substituted with alkyl, amino, mono or dialkylaminoalkyl, trialkylammoniumalkyl, or may be optionally quaternized on the nitrogen with an alkyl group;

R^2 is selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkyl, C_{3-8} cycloalkyl, aryl, aryl(C_{1-2} alkyl), hydroxyalkyl, alkoxyalkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, trialkylammoniumalkyl, alkylcarbonyl, arylcarbonyl, alkylsulfonyl, arylsulfonyl, alkylenecarboxylate, alkylenecarboxamide, alkylenesulfonate, and alkylenesulfonic acid;

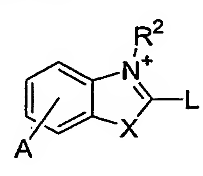
R^5 is selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, monoalkylamino, dialkylamino, trialkylammonium, dialkylaminoalkyl, and trialkylammoniumalkyl, aryl, heteroaryl, each of which may be optionally substituted;

R^8 is selected from the group consisting of hydrogen, halogen, alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, alkenyl, polyalkenyl, alkynyl, polyalkynyl, alkenylalkynyl, aryl, heteroaryl, alkoxy, alkylthio, and dialkylamino, each of which may be optionally substituted; an acyclic heteroatom-containing moiety or a cyclic heteroatom-containing moiety; a BRIDGE-DYE; and a reactive group;

X is oxygen or sulfur; and

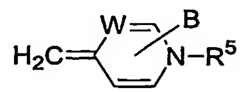
L is a leaving group.

77. The process of claim 76 wherein the reacting step includes reacting a compound having the formula:



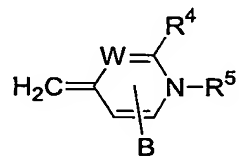
wherein L is halo, alkylthio, arylthio, alkylsulfonyl, or arylsulfonyl.

78. The process of claim 76 wherein the reacting step includes reacting a compound having the formula:



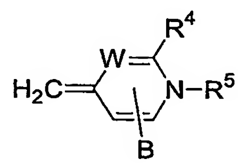
wherein R^5 is C_{2-6} alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, monoalkylamino, dialkylamino, trialkylammonium, dialkylaminoalkyl, and trialkylammoniumalkyl, aryl, heteroaryl, each of which may be optionally substituted.

79. The process of claim 76 wherein the reacting step includes reacting a compound having the formula:



wherein R^4 is selected from the group consisting of halo, alkylthio, arylthio, alkylsulfonyl, and arylsulfonyl.

80. The process of claim 79 further comprising the step of reacting a compound having the formula:



with a nucleophile capable of displacing the group R^4.

81. The process of claim 79 wherein W is -CH=.

82. The process of claim 76 where R^5 is selected from the group consisting of C_{2-6} alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, monoalkylamino, dialkylamino, trialkylammonium, dialkylaminoalkyl, and trialkylammoniumalkyl, aryl, heteroaryl, each of which may be optionally substituted.